



Proteomic and metabolic profiles of *Beta vulgaris* root tips: changes induced in response to iron deficiency and resupply



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Background

Iron-deficient Strategy I plants develop a series of biochemical and morphological changes in roots that lead to an increased capacity for Fe uptake. These responses at the uptake level are accompanied by several metabolic changes that support this adaptation mechanism to Fe-deficiency [1]. These metabolic changes include an induction of carbon metabolism pathways with a increase in the activities of PEPC and several enzymes of the glycolytic pathway and the TCA cycle. These changes can be partially reverted after Fe resupply [2, 3]. Other changes include accumulation of organic acids and flavins and excretion of phenolic compounds and flavins. In this work, a comprehensive analysis of the metabolic and proteomic changes observed in sugar beet root tips with Fe-deficiency and Fe-resupply has been carried out.

Experimental

□ ROOT TIPS were taken from Fe-sufficient (grown with 45 μ M Fe(III)-EDTA), Fe-deficient (grown with 0 μ M Fe), and Fe-resupplied *Beta vulgaris* plants (24 and 72 hours after Fe resupply with 45 μ M Fe(III)-EDTA).

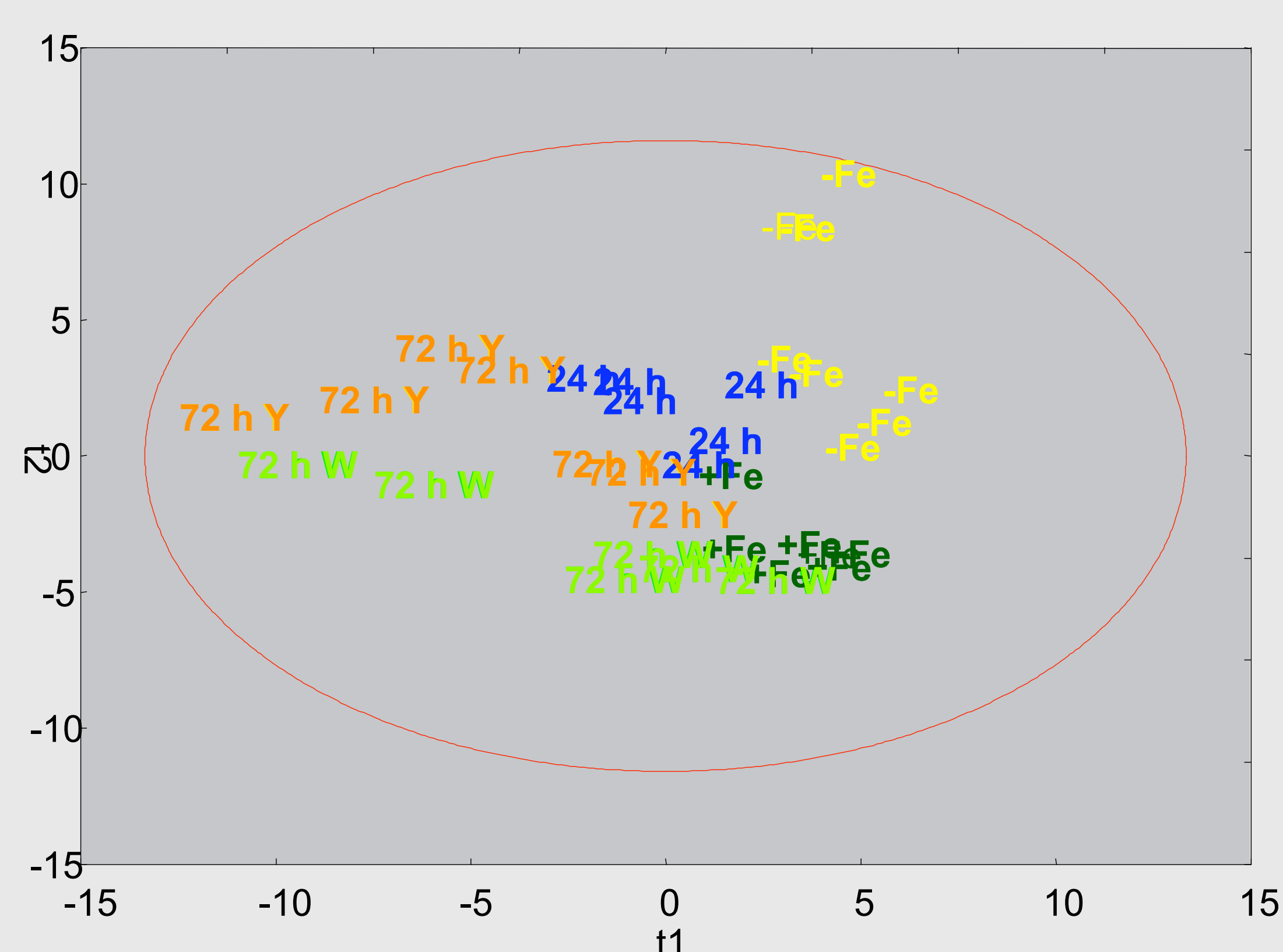
□ METABOLOMIC PROFILES: extracts were obtained as described elsewhere [5]. A mixture of internal retention index markers composed by different fatty acid markers were added to the dried extracts. Samples were derivatized in two steps with methoxiamine hydrochloride and MSTFA 1%, randomized and analyzed by GC-MS following the recommendations described by the Metabolomics Standards Initiative [3]. Mass chromatograms were deconvoluted using the Leco ChromaTOF software and peaks were exported to the BinBase database [5] and identified using the Fiehn Library (<http://fiehnlab.ucdavis.edu/Metabolite-Library-2007>).

□ PROTEOMIC PROFILES: extracts were obtained according to Meyer *et al.* [4] and run in 2D gels. The first dimension was run with a pI linear gradient from 5 to 8 and focused at 20 °C for a total of 14000 V.h. For the second dimension IPG strips were loaded into 12% SDS-PAGE and run at 20 mA for 1.5 h. Gels were stained with Coomassie blue and analyzed with the Bio-Rad PD Quest 8.0 software. Protein spots were excised using a Proteiner DP protein station and analyzed using a Bruker Ultraflex MALDI TOF-TOF and LIFT TOF-TOF. Peptide masses were used as input to search the NCBI database using Mascot Software.

□ EXPRESSION OF 6,7-DIMETHYL-8-NBITYLLUMAZINE (DMRL) SYNTHASE transcripts in root tips was analyzed by semi-quantitative RT-PCR.

Metabolomic profiles

FIGURE 1. Score scatter plot of component 1 vs. component 2 after Partial Least Square analysis of identified metabolites. Fe sufficient (+Fe), Fe-deficient (-Fe), 24 Fe-resupplied (24h) and 72 h Fe-resupplied root tip yellow zone (72 h Y) and new white zone (72 h W). The red circle contains a \pm 3.00 STD Dev.

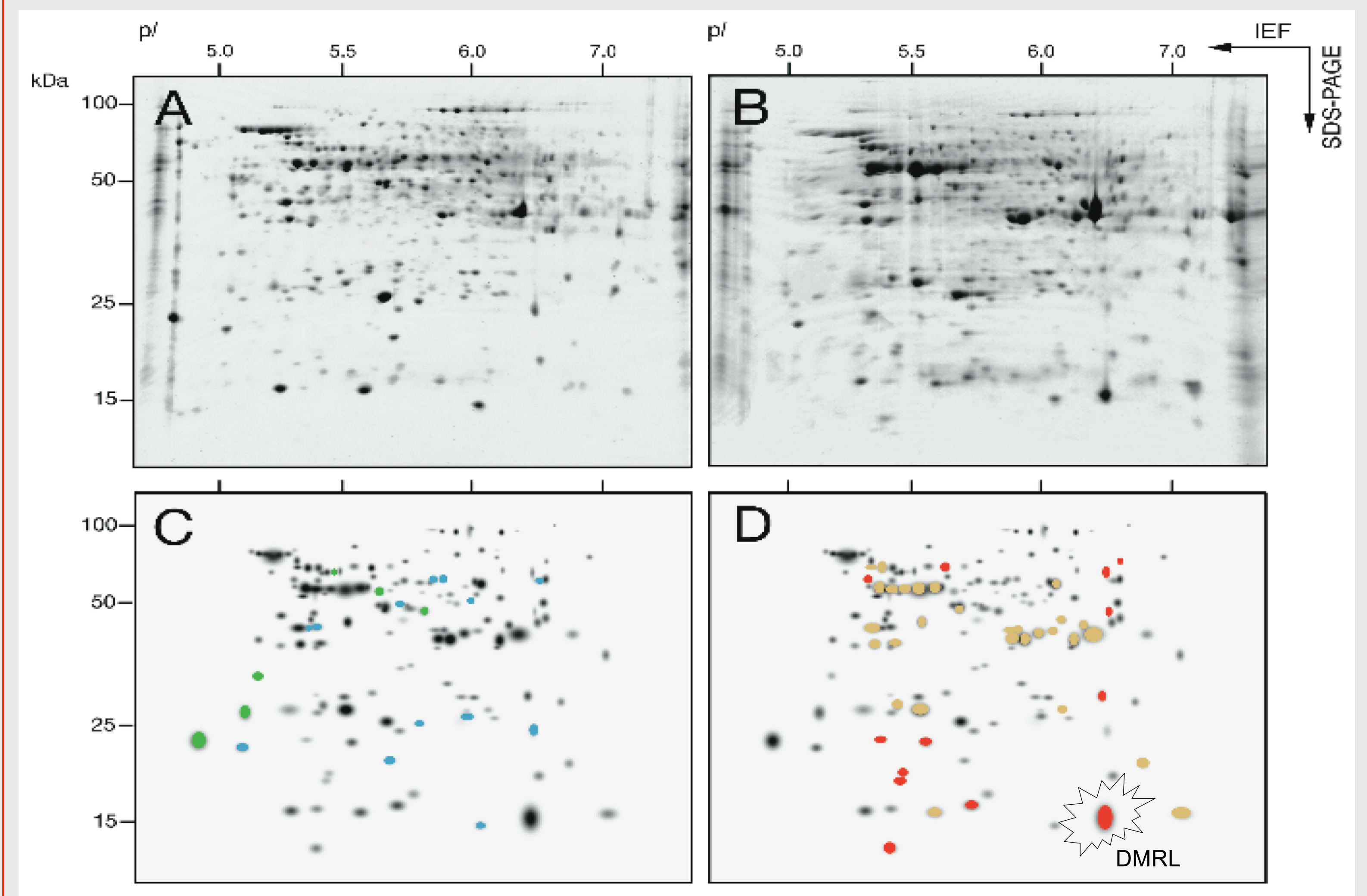


□ Seventy six metabolites were identified in root tips. A principal component analysis of the identified metabolites shows a good separation between Fe-sufficient and Fe-deficient root tips. 24 h Fe-resupplied root tip metabolites fall between those of Fe-deficient and Fe-sufficient root tips. 72 h Fe-resupplied root tip metabolites show a larger degree of variation due to sampling difficulties.

□ An increase in organic acid metabolism (TCA cycle) with Fe-deficiency was observed in agreement with previous results [1]. An activation of the raffinose series of oligosaccharides (RSOs), including raffinose, galactinol and myo-inositol was also observed in Fe-deficient and 24-h resupplied plants. This activation has never been described before in plants under Fe deficiency, although is a common response in plants under other stresses.

Proteomic profiles

FIGURE 2. 2-D IEF-SDS PAGE proteome maps of root tips from Fe-sufficient (A & C) and Fe-deficient (B & D) plants. Scans of real typical gels are shown in A and B. Virtual composite images (C & D) created containing all spots present in the real gels. Spots whose intensities decrease or disappear completely with Fe deficiency are labeled with blue and green marks, respectively (C), and those increasing with Fe deficiency or only present in Fe-deficient gels are labeled with orange and red marks, respectively (D).



□ Fe-deficiency results in relative intensity changes in a large number of these proteins. Most of the proteins found to be up-regulated by Fe deficiency were identified as carbohydrate catabolism enzymes, including 5 out of 10 enzymes of the glycolytic pathway. Fe-deficiency also increased the activities of several enzymes of the citric acid cycle. Up-regulation of carbohydrate catabolism in roots of plants grown in Fe deficient conditions is probably a result of an increased demand of energy and reducing power in roots needed to sustain the increased activity of H⁺-ATPase and Fe-reductase.

Expression of DMRL synthase

FIGURE 3. Semi-quantitative RT-PCR analysis of the BvDMRLs transcript in root tip extracts. Fe sufficient (+Fe), Fe-deficient (-Fe), 24 Fe-resupplied (24h) and 72 h Fe-resupplied root tip yellow zone (72 h yellow) and new white zone (72 h white).



□ The largest change found in the proteome map of root tip extracts from plants grown in Fe-deficiency corresponds to appearance of a spot highlighted in Fig 2D and identified as DMRL synthase. The expression of DMRL synthase was also up-regulated in Fe-deficient conditions.

Conclusions

□ Fe-deficiency increased the amount of many TCA cycle intermediates in *Beta vulgaris* root tips. An increase in the amount of the raffinose series of oligosaccharides, including raffinose, galactinol and myo-inositol was observed in Fe-deficient and 24 hours Fe-resupplied plant roots.

□ Fe-deficiency induced significant intensity changes in a large number of proteins, many of them associated to carbohydrate catabolism. A protein identified as DMRL synthase was very abundant in root tips from Fe-deficient sugar beet and was not detectable in Fe-sufficient roots. This protein was found to be regulated transcriptionally by Fe status.

References

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Acknowledgments

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